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(54) Title: METHOD FOR PRODUCTION OF A KAPPA-CASEIN GLYCOMACROPEPTIDE AND USE OF A KAPPA-CASEIN GLYCOMACROPEPTIDE

(57) Abstract

The method utilizes whey as a starting material, followed by specified reaction steps including ultrafiltration and heat treatment. The Kappa-casein glycomacropeptide can be used as a part of the diet for specified patients and as a medicament against diarrhea caused by viral infection in the intestines.

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METHOD FOR PRODUCTION OF A KAPPA-CASEIN GLYCOMACROPEPTIDE AND USE OF A KAPPA-CASEIN GLYCOMACROPEPTIDE

The invention comprises a method for production of a Kappa-casein glycomacropeptide, in the following usually abbreviated as GMP, and a use of a GMP.

GMP has important utilities in different fields, *vide* EP-A1 488589, col. 1, lines 32-45. Thus, there is a need for a cheap and simple method for production of GMP.

EP 488589 describes a method for production of GMP. This prior art method uses an ion exchanger, and thus the tedious and time consuming process comprising regeneration of this ion exchanger is part of the prior art process.

In Milchwissenschaft 47(10) 1992, pages 615-619 another method for production of GMP is described. This prior art method involves the step of protein precipitation by means of trichloro acetic acid. The difficulty relating to the subsequent removal of the residual trichloro acetic acid from the resulting GMP is a serious drawback in relation to this prior art method.

Thus, it is the purpose of the invention to provide a method for production of a GMP, which method is cheaper and simpler than the prior art methods.

The method according to the invention for production of a Kappa-casein glycomacropeptide is characterized by the fact that

1) GMP containing whey is used as a starting material,
2) the proteins in this starting material are isolated in the retentate in an ultrafiltration equipment with membranes with a cut-off value as large as possible which will retain the whey proteins and the GMP in the retentate,
3) the retentate from step 2) is heat treated to cause denaturation of the whey proteins,
4) the heat treated retentate from step 3) is acidified to a pH value in the vicinity of the isoelectric point of the whey proteins, and
5) the precipitate generated during step 4) is separated from the supernatant/filtrate, which is collected as a solution of the GMP.

Surprisingly it has been found that GMP by use of the method according to the invention can be produced on the basis of the very cheap GMP containing whey as a starting material and by means of few and simple process steps, in high yield and in high purity. In this specification with claims GMP containing whey is normally either whey from cheese production or whey from production of rennet precipitated casein. Both these kinds of whey are cheap and ordinarily considered as waste products.

Also, surprisingly it has been found that the GMP produced by means of the method according to the invention is free of or almost free of phenylalanine. As the GMP produced by means of the method according to the invention surprisingly can also be converted to an edible material, it can be used for PKU patients (PKU is phenylketonurea) as a part of their diet, *vide* later in this specification for a more detailed description thereof.

From Food Australia 43 (6), June 1991, pages 252-254 it appears that a phenylalanine free GMP and the use thereof as a food constituent for PKU patients is described. This prior art method uses an anion exchange resin, and thus the tedious and time consuming process comprising regeneration of this anion exchange resin is part of the prior art process.

In US 5,061,622 another method for production of GMP is described. In this method isolated casein is used as the starting material. Due to the fact that no heat treatment is used during this prior art method, it is impossible to use a whey containing product as a starting material, in contradistinction to the method according to the invention.

It is admitted that it appears from Japanese unexamined application no. 3294299 that GMP can be produced on the basis of a whey protein containing solution. However, this prior art method comprises as an imperative step a freezing and thawing process. Freezing equipment is not a normal part of a dairy, and also, freezing is a process requiring a large amount of energy. In contradistinction thereto, the method according to the invention can be carried out with equipment which is an ordinary part of a dairy, and with low energy costs.

Also, Japanese unexamined application no. 1168693 comprising preparation of sialic acid describes the use of whey as a starting material,

coagulation of whey proteins and ultrafiltration of the supernatant. Even if this prior art method can also be used for production of GMP, the purity of the GMP is low.

A preferred embodiment of the method according to the invention is characterized by the fact that the starting material is whey from rennet coagulated milk. This starting material is extremely cheap and thus provides an unusually cheap end product.

A preferred embodiment of the method according to the invention is characterized by the fact that the cut-off value of the membranes in the ultrafiltration equipment used in step 2) is 16,000 - 20,000 Dalton. It has been found that this embodiment provides a sharp separation between the whey proteins and the GMP (which both stay in the retentate) and the low molecular lactose and salts (which are transferred to the permeate).

A preferred embodiment of the method according to the invention is characterized by the fact that the pH value in step 4 is 4 - 5. It has been found that hereby a complete precipitation of the denatured whey proteins takes place, whereas the GMP stays in solution.

A preferred embodiment of the method according to the invention is characterized by the fact that the separation in step 5) is performed by means of an ultrafiltration. In this manner the GMP is found in the permeate in a very pure condition.

A preferred embodiment of the method according to the invention is characterized by the fact that the pH of the supernatant/filtrate of step 5) is adjusted to a value depending upon the future use of the GMP. If for instance the GMP solution is intended for use as a constituent in an artificial milk comprising also milk fat and lactose, the pH should be adjusted to around neutrality. This artificial milk would be free of phenylalanine and thus be well suited for PKU patients.

Also, the invention comprises a use of the GMP preparable according to the invention as a part of the diet for PKU patients. The phenylalanine free GMP used so far by PKU patients has been expensive, *vide* the above remarks in relation to Food Australia 43 (6), June 1991, pages 252-254. According to the invention the cost of the nitrogeneous intake for PKU patients can be heavily reduced.

The GMP can be decomposed, enzymatically and by other means, to GMP fractions. The proportion between the sugar parts and the protein parts in these GMP fractions can differ from the proportion between the sugar parts and the protein parts in the GMP. It is to be understood that the scope of this patent will also cover a method for production of such GMP fractions and a use of such GMP fractions.

EXAMPLE 1

Lacprodan-80 is a commercial product comprising a whey concentrate and produced as indicated in the product sheet Lacprodan-80 PI 9013999E/09.91 from Danmark Protein A/S, DK-6920 Videbaek, Denmark, and a slurry of Lacprodan-80 with a protein content of 8% is prepared (step 1) and 2)). This slurry is heat treated at 95°C for 15 minutes (step 3)) and then cooled to 50°C. The pH value of this slurry is adjusted to 4.5 with HCl (step 4)). Subsequently a filtration comprising a GF/D filter (Whatman) followed by a GF/F filter (Whatman) (step 5)) is performed. The filtrate contained the GMP whereby the protein part of the GMP constituted 70% of the total amount of protein.

EXAMPLE 2

Lacprodan-80 is a commercial product comprising a whey concentrate and produced as indicated in the product sheet Lacprodan-80 PI 9013999E/09.91 from Danmark Protein A/S, DK-6920 Videbaek, Denmark, and a slurry of Lacprodan-80 with a protein content of 8% is prepared (step 1) and 2)). This slurry is heat treated at 95°C for 15 minutes (step 3)) and then cooled to 50°C. The pH value of this slurry is adjusted to 4.5 with HCl (step 4)). Subsequently an ultrafiltration by means of PCI membranes with a cut off value of 100,000 Dalton was performed (step 5)). The permeate is concentrated to ° Brix = 10 by hyperfiltration (DDS module). The concentrate is spray dried. The protein constituted 53.4% of the dry

matter. The phenylalanine content of the protein is around 1/3 of the phenylalanine content in the raw material, which is considered satisfactory.

EXAMPLE 3

This experiment is performed exactly as Example 2 with the exception that a supplementary centrifugation is carried out immediately before the ultrafiltration. In this manner the ultrafiltration can be performed with a higher flux.

EXAMPLE 4

A milk like product for PKU patients was formulated. The recipe was as follows:

Glycomacropeptide product from Example 2	35.25 g
Butter oil from unsalted butter	18.00 -
Lactose	20.00 -
Water	426.75 -

The composition of the milk like product was as follows:

Protein	3.5%
Fat	3.6%
Lactose	4.0%

The mixture was mixed and homogenized (Rannie homogenizer, 40°C, 250 bar).

The flavor of the milk like product was pleasant and well tasting, very similar to milk. Creaming was observed after approx. 30 minutes though no fat separation occurred even after 24 hours at 5°C.

EXAMPLE 5

A yoghurt like product for PKU patients was made from the milk like product in Example 4.

250 g of the milk like product was added to 10 g of maize starch.

To the mixture, which was heated to 80°C for 1 minute, and subsequently cooled to 43°C was added yoghurt starter culture (YC-380, Chr. Hansens laboratory) in a dosage of 0.02%.

pH start: 6.24; pH after 6 hours: 4.6.

The product appeared to exhibit a too high viscosity for a yoghurt, although the flavor was good. The product was found very useful as a dessert like product.

CLAIMS

1. Method for production of a Kappa-casein glycomacropeptide, characterized by the fact that

1) Kappa-casein glycomacropeptide containing whey is used as a starting material,

2) the proteins in this starting material are isolated in the retentate in an ultrafiltration equipment with membranes with a cut-off value as large as possible which will retain the whey proteins and the Kappa-casein glycomacropeptide in the retentate,

3) the retentate from step 2) is heat treated to cause denaturation of the whey proteins,

4) the heat treated retentate from step 3) is acidified to a pH value in the vicinity of the isoelectric point of the whey proteins, and

5) the precipitate generated during step 4) is separated from the supernatant/filtrate, which is collected as a solution of the Kappa-casein glycomacropeptide.

2. Method according to Claim 1, characterized by the fact that the starting material is whey from rennet coagulated milk.

3. Method according to Claims 1 - 2, characterized by the fact that the cut-off value of the membranes in the ultrafiltration equipment used in step 2) is 16,000 - 20,000 Dalton.

4. Method according to Claims 1 - 3, characterized by the fact that the pH value in step 4 is 4 - 5.

5. Method according to Claims 1 - 4, characterized by the fact that the separation in step 5) is performed by means of an ultrafiltration.

6. Method according to Claims 1 - 5, characterized by the fact that the pH of the supernatant/filtrate of step 5) is adjusted to a value depending upon the future use of the Kappa-casein glycomacropeptide.

7. Use of the Kappa-casein glycomacropeptide preparable according to Claims 1 - 6, characterized by the fact that the Kappa-casein glycomacropeptide is used as a part of the diet for phenylketonurea patients.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00013

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C07K 3/26, A23J 1/20, A61K 37/16
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C07K, A23J, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A2, 0393850 (SNOW BRAND MILK PRODUCTS CO., LTD.), 24 October 1990 (24.10.90), see column 2, line 30 - column 4, line 29, claims and the whole document	1-7

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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INTERNATIONAL SEARCH REPORT
Information on patent family members

26/02/94

International application No.
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0393850	24/10/90	DE-U- 6900072	18/02/93
		JP-A- 2276542	13/11/90
		US-A- 5075424	24/12/91